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REMARKS

Reconsideration of the present application in view of the above amendments and the following remarks is respectfully requested. Claims 1-7 and 9-22 were pending, of which claims 14 and 15 were withdrawn from further consideration. Claims 1, 5, 16, 19, and 20 have been amended. Support for the language "physiologically acceptable" in claims 1, 5, 16 and 20 may be found at page 3, lines 6-9. Additional support for the amendments in claim 16 may be found at page 5, lines 10-19. Support for the amendments in claim 19 may be found at page 5, lines 10-14. No new matter has been added.

Formal Matters:

The title of the invention stands objected to as allegedly not descriptive. More specifically, the Examiner states that (a) EDTA is required by all claims, and (b) it is the presence of EDTA that distinguishes the claimed invention from the prior art.

While believing that the title proposed in the amendment filed August 15, 2003 is descriptive, to facilitate allowance, Applicants have amended the title to "USE OF EDTA IN STABILIZING GRANULOCYTE MACROPHAGE COLONY-STIMULATING FACTOR." Accordingly, Applicants respectfully submit that this ground of objection has been overcome.

Rejection Under 35 U.S.C. §112, Second Paragraph

Claim 19 stands rejected under 35 U.S.C. § 112, second paragraph, for allegedly being indefinite. More specifically, the Action states that this claim recites the amounts of mannitol, sucrose and TRIS as masses only and not as concentrations.

Applicants thank the Examiner for noting the above informalities. Applicants have amended claim 19 by inserting "/ml" as suggested by the Examiner. Accordingly, Applicants respectfully submit that this ground of rejection has been overcome.

Rejection Under 35 U.S.C. § 103(a)

Claims 1-7 and 9 stand under 35 U.S.C. §103(a) as being unpatentable over the LEUKINE® Sargramostim product insert, in view of Chalmers, Manufacturing Chemist & Aerosol News (March 1978, cited by Applicants), and U.S. Patent Number 5,217,954 (Foster *et al.*), and in the case of claims 4-7, further in view of U.S. Patent Number 5,545,536 (Kaushansky *et al.*), for the reasons set forth in the previous Office Action (Paper No. 11 dated May 15, 2003). The Action deems unpersuasive the arguments in the previous response (filed on August 15, 2003) that no motivation to combine the cited references was present in prior art. The Action asserts that the motivation to combine the cited references may be found in the LEUKINE® Sargramostim product insert, which warns against administering benzyl alcohol to newborns. In addition, the Action asserts that the motivation to combine the cited references may also be found in Foster *et al.*, which describes the use of EDTA to stabilize a cytokine (*i.e.*, bFGF) preparation.

Applicants respectfully traverse this ground of rejection. Applicants submit that a *prima facie* case of obviousness has not been established by the Action: there is not sufficient motivation for one of ordinary skill in the art to combine the cited references. The warning against administering benzyl alcohol to neonates in the LEUKINE® Sargramostim product insert does not provide the necessary motivation for substituting benzyl alcohol with EDTA in a GM-CSF formulation. First, the product insert describes not only LEUKINE formulations that contain benzyl alcohol, but also a LEUKINE formulation that does not contain benzyl alcohol. More specifically, the product insert describes that lyophilized LEUKINE may be constituted with 1 mL sterile water for injection (*see*, the second paragraph on page 1 of the insert). Thus, one of ordinary skill in the art, in view of the insert as a whole, would use lyophilized LEUKINE reconstituted in sterile water for administering to neonates. Such an approach would avoid significant time and efforts required for developing a new formulation.

Even assuming for the sake of argument that one is motivated to substitute benzyl alcohol with another antimicrobial preservative for administering GM-CSF in neonates, EDTA would not be an obvious choice. Generally a substance must meet the requirements of

appropriate regulatory guidelines regarding Antimicrobial Effectiveness Testing (AET) and Preservative Effectiveness Testing (PET) to be considered for use as a preservative. There is no evidence in the art that EDTA meets such requirements. Furthermore, many other antimicrobial preservatives (*e.g.*, phenol) have been used in the industry, some of which are suitable for administering to neonates. Accordingly, if one of ordinary skill in the art were to replace benzyl alcohol, such a person would choose a substance known to be suitable for administrating to neonates as a preservative instead of EDTA.

Applicants further submit that the Foster *et al.* reference also fails to provide the necessary motivation for using EDTA to stabilize GM-CSF. That both bFGF and GM-CSF are cytokines is insufficient for motivating one of ordinary skill in the art to stabilize GM-CSF with EDTA. The term "cytokine," as known in the art, refers to extracellular signal protein or peptide that acts as a local mediator in cell-cell communication (*see*, *Molecular Biology of The Cell*, 4th Ed. Alberts *et al.*, ed., published Garland Science, 2002, p. G:10, copy enclosed). It encompasses a diverse group of proteins and peptides with different amino acid sequences and functions. In fact, bFGF and GM-CSF are unrelated in their amino acid sequences: They only share about 10% sequence identity. As one of ordinary skill in the art would appreciate that a primary factor that determines the level of protein degradation is the amino acid sequence of the protein of interest. Accordingly, Applicants believe that such a person, in view of the sequence differences between bFGF and GM-CSF, would not have been motivated to use EDTA to stabilize GM-CSF.

Even assuming for the sake of argument that a *prima facie* case of obviousness has been established, Applicants respectfully submit that such a *prima facie* case of obviousness can be rebutted by the unexpected results disclosed in the present application. For instance, Examples 2 and 3 show that EDTA reduces N-terminal degradation of GM-CSF. Such results are unexpected in view of the cited references. None of the cited references suggest that the claimed GM-CSF formulation would have no, or a reduced level of, N-terminal degradation. The Foster *et al.* reference only discloses that EDTA may reduce the oxidation or metal-induced

In view of the above remarks, Applicants submit that this ground of rejection under 35 U.S.C. § 103(a) has been overcome. Withdrawal of this rejection is respectfully requested.

Claims 10-13 stand rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over the LEUKINE® Sargramostim product insert, Chalmers, Foster, and further in view of U.S. Patent Number 6,500,418 B1 (Dieckgraefe *et al.*) for reasons set forth in the previous Office Action (Paper No 11).

Applicants respectfully traverse this ground of rejection. As discussed above, the composition of claim 1 is not obvious in view of the LEUKINE® Sargramostim product insert, Chalmers, and Foster for failing to provide the necessary motivation for using EDTA to stabilize GM-CSF. Such a deficiency has not been remedied by the Dieckgraefe *et al.* reference. More specifically, the Dieckgraefe *et al.* reference relates to the use of GM-CSF in treating inflammatory bowel disease. It does not suggest or teach the use of EDTA in stabilizing GM-CSF. Thus, Applicants submit that the methods of using the composition of claim 1, as recited in claims 10-13, would not be deemed obvious in light of the Dieckgraefe *et al.* reference.

In view of the above remarks, Applicants submit that this ground of rejection has been overcome. Withdrawal of this rejection is respectfully requested.

The Director is authorized to charge any additional fees due by way of this Amendment, or credit any overpayment, to our Deposit Account No. 19-1090.

All of the claims remaining in the application are believed to be allowable. Favorable consideration and a Notice of Allowance are earnestly solicited.

Respectfully submitted,

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Enclosures:

Postcard
Copy of *Molecular Biology of The Cell*, 4th Ed. Alberts *et al.*, ed., published Garland Science, 2002, p. G:10

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MOLECULAR BIOLOGY OF THE CELL

fourth edition

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Front cover Human Genome: Reprinted by permission from *Nature*, International Human Genome Sequencing Consortium, 409:860–921, 2001 © Macmillan Magazines Ltd. Adapted from an image by Francis Collins, NHGRI; Jim Kent, UCSC; Ewan Birney, EBI; and Darryl Leja, NHGRI; showing a portion of Chromosome 1 from the initial sequencing of the human genome.

Back cover In 1967, the British artist Peter Blake created a design classic. Nearly 35 years later Nigel Orme (illustrator), Richard Denyer (photographer), and the authors have together produced an affectionate tribute to Mr Blake's image. With its gallery of icons and influences, its assembly created almost as much complexity, intrigue and mystery as the original. *Drosophila, Arabidopsis,* Dolly and the assembled company tempt you to dip inside where, as in the original, "a splendid time is guaranteed for all." (Gunter Blobel, courtesy of The Rockefeller University; Marie Curie, Keystone Press Agency Inc; Darwin bust, by permission of the President and Council of the Royal Society; Rosalind Franklin, courtesy of Cold Spring Harbor Laboratory Archives; Dorothy Hodgkin, © The Nobel Foundation, 1964; James Joyce, etching by Peter Blake; Robert Johnson, photo booth self-portrait early 1930s, © 1986 Delta Haze Corporation all rights reserved, used by permission; Albert L. Lehninger, (unidentified photographer) courtesy of The Alan Mason Chesney Medical Archives of The Johns Hopkins Medical Institutions; Linus Pauling, from Ava Helen and Linus Pauling Papers, Special Collections, Oregon State University; Nicholas Poussin, courtesy of ArtToday.com; Barbara McClintock, © David Micklos, 1983; Andrei Sakharov, courtesy of Elena Bonner; Frederick Sanger, © The Nobel Foundation, 1958.)

cyclic AMP (cAMP)
Nucleotide that is generated from ATP by adenylyl cyclase in response to stimulation of many types of cell-surface receptors. cAMP acts as an intracellular signaling molecule by activating cyclic-AMP-dependent kinase (protein kinase A, PKA). It is hydrolyzed to AMP by a phosphodiesterase.

cyclic AMP-dependent protein kinase (protein kinase A, PKA)
Enzyme that phosphorylates target proteins in response to a rise in intracellular cyclic AMP.

cyclic GMP
Small soluble intracellular signaling molecule formed from GTP by the enzyme guanylyl cyclase in response to photoreceptor stimulation in the retina.

cyclin
Protein that periodically rises and falls in concentration in step with the eucaryotic cell cycle. Cyclins activate crucial protein kinases (called a cyclin-dependent protein kinase, or Cdk) and thereby help control progression from one stage of the cell cycle to the next.

cyclin-Cdk complex
Protein complexes that are formed periodically during the eucaryotic cell cycle as the level of cyclin increases, and in which the cyclin-dependent kinase (Cdk) becomes partially activated.

cyclin-dependent kinase (Cdk)
Protein kinase that has to be complexed with a cyclin protein in order to act. Different Cdk-cyclin complexes trigger different steps in the cell-division cycle by phosphorylating specific target proteins.

cytochrome b-c_1 complex
Second of the three electron-driven proton pumps in the respiratory chain. It accepts electrons from ubiquinone.

cytochrome oxidase complex
Third of the three electron-driven proton pumps in the respiratory chain. It accepts electrons from cytochrome *c* and generates water using molecular oxygen as an electron acceptor.

cytochrome
Colored, heme-containing protein that transfers electrons during cellular respiration and photosynthesis.

cytokine
Extracellular signal protein or peptide that acts as a local mediator in cell–cell communication.

cytokine receptor
Type of cell-surface receptor whose ligands are cytokines such as interferons, growth hormone and prolactin, and which acts through the Jak-STAT pathway.

cytokinesis
Division of the cytoplasm of a plant or animal cell into two, as distinct from the division of its nucleus (which is mitosis).

cytoplasm
Contents of a cell that are contained within its plasma membrane but, in the case of eucaryotic cells, outside the nucleus.

cytoskeleton
System of protein filaments in the cytoplasm of a eucaryotic cell that gives the cell shape and the capacity for directed movement. Its most abundant components are actin filaments, microtubules, and intermediate filaments.

cytosol
Contents of the main compartment of the cytoplasm, excluding membrane-bounded organelles such as endoplasmic reticulum and mitochondria. Originally defined operationally as the cell fraction remaining after membranes, cytoskeletal components, and other organelles have been removed by low-speed centrifugation.

cytotoxic T cell
Type of T cell responsible for killing infected cells.

$\Delta G°$—*see* standard free-energy change

ΔG—*see* free-energy change

dalton
Unit of molecular mass. Approximately equal to the mass of a hydrogen atom (1.66×10^{-24} g).

default pathway
Constitutive secretory pathway that automatically delivers material from the Golgi apparatus to the plasma membrane if no other sorting signals are present.

degenerate
Not a moral judgment but an adjective that describes multiple states that amount to the same thing: different triplet combinations of nucleotide bases (codons) that code for the same amino acid, for example.

deletion
Type of mutation in which a single nucleotide or sequence of nucleotides has been removed from the DNA.

denaturation
Dramatic change in conformation of a protein or nucleic acid caused by heating or by exposure to chemicals and usually resulting in the loss of biological function.

dendrite
Extension of a nerve cell, typically branched and relatively short, that receives stimuli from other nerve cells.

dendritic cell
Cell derived from bone marrow and present in lymphoid and other tissues that is specialized for the uptake of particulate material by phagocytosis and which acts as a "professional" antigen-presenting cell in immune responses.

deoxyribonucleic acid—*see* DNA

desensitization—*see* adaptation

desmosome
Type of anchoring cell–cell junction, usually formed between two epithelial cells, characterized by dense plaques of protein into which intermediate filaments in the two adjoining cells insert.

detergent
Type of small amphipathic molecule that tends to coalesce in water, with its hydrophobic tails buried and its hydrophilic heads exposed. It is widely used to solubilize membrane proteins.

determined
In developmental biology, an embryonic cell is said to be determined if it has become committed to a particular specialized path of development. This **determination** reflects a change in the internal character of the cell, and it precedes the much more readily detected process of cell differentiation.

development
Succession of changes that take place in an organism as a fertilized egg gives rise to an adult plant or animal.

diacylglycerol
Lipid produced by the cleavage of inositol phospholipids in response to extracellular signals. Composed of two fatty acid chains linked to glycerol, it serves as a signaling molecule to help activate protein kinase C.